This is the first publication describing the creation of human embryonic stem cell lines. The researchers obtained fresh or frozen human cleavage stage (2-16 cell stages) embryos produced during in vitro fertilization treatment. Individuals donating their embryos provided informed consent. The embryos were allowed to develop to the blastocyst stage. The inner cell mass of 14 embryos were isolated and fives cell lines were derived.

The article defines three essential features of embryonic stem cells. 1) Derived from an early stage embryo 2) the cells proliferate for an extended period of time but remain undifferentiated and 3) the cells retain the ability to differentiate into all three embryonic germ layers. The authors showed they accomplished the first criterion. The rest of the article validates that the cells are **pluripotent** stem cells by examining the last two criteria.

They first analyze the ability of the cell lines to proliferate for extended periods of time in an undifferentiated state. Four of the five cell lines were maintained for 5 to 6 months and then frozen for preservation. The last cell line was being continuously cultured at the time of publication. At no point did the cells appear to stop proliferating. All the cell lines had a normal karyotype. A karotype describes the chromosomes in a particular cell. Normal human cells have 23 pairs of chromosomes, including a pair of sex chromosomes. When embryonic stem cells are grown in culture they can develop an abnormal number of chromosomes, a condition called **aneuploidy**. Aneuploidy would indicate that there had been abnormal cell division and the cells were not healthy.

Another indication that cells have the ability to divide indefinitely is the presence of telomerase. A **telomere** is a protective, non-coding piece of DNA located at the tips of chromosomes. Each time the DNA is copied during cell division (this happens thousands of times on the developmental journey from embryonic stem cells to adult cells), a small piece of the telomere is lost. When the telomere becomes too short the coding DNA cannot be copied correctly and cell division halts. **Telomerase** is an enzyme that restores the telomeres and lets the cell keep dividing. The cell lines were found to express high amounts of telomerase, which would suggest that the cells are able to divide forever (in the right conditions).

To test whether the cells are undifferentiated they perform antibody staining against proteins that are known to be expressed in pluripotent *non-human* primate cells. They found that the cells were positive for pluripotency markers SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81.

Next they examine the third criterion, the ability of the cells to differentiate into all three germ layers. In non-human animals, this ability has been tested by creating chimeras. A chimera is a single animal that has cells from more than one genetic background. To form a chimera, ES cells are injected into another embryo of the same species. The embryo is implanted and allowed to develop and be born. The resulting animal has differentiated cells that are genetically identical to the original embryo as well as differentiated cells that are genetically identical to the injected ES cells.

Another test the authors used to determine the stem cells' ability to differentiate into tissues from all three germ layers was **teratoma** formation. As this is an unethical procedure to perform on human beings, scientists instead observe the behavior of the cells in mice. The ES cells are injected in a SCID mouse. A SCID mouse has a mutation that causes an immune deficiency, and therefore the mouse will not reject the foreign cells. Once injected the ES cells form tumors called teratomas. The teratomas were stained and observed to contain differentiated cells from all three germ layers.

The paper concludes with discussing the potential uses of human embryonic stem cells, including researching early human development and potential stem cell therapies.